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A STUDY OF POSSIBLE TOXIC EFFECTS OF CARBAMATE FINISHING AGENTS

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CONTENTS

	Page
Abstract	1
Introduction	1
Background	2
Previous pharmacological work with carbamates	3
Experimental results	4
Rabbit skin and eye irritation tests	
Mouse lung-tumor tests	
Two-year rat feeding study of carbamate derivatives	
Materials and methods	
Results	
Histopathology	
Discussion of rat feeding studies	
Summary	10
Acknowledgment	11
Literature cited	15

A STUDY OF POSSIBLE TOXIC EFFECTS OF CARBAMATE FINISHING AGENTS

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ABSTRACT

Dimethylol alkyl carbamates of the type used as textile finishing agents, together with the parent carbamates and appropriate control agents, have been evaluated toxicologically: (a) As skin irritants, (b) as producers of mouse lung tumors, and (c) for toxic effects in long-term feeding of rats.

No skin irritation was observed when treated cotton cloth (uncured) was patch-tested on rabbit. Under long-continued and severe conditions, when 50-percent alcoholic solutions were applied, methyl carbamates and the dimethylol derivatives of methyl, ethyl, and hydroxyethyl carbamates were moderate irritants about equal in effect to that of 5-percent formaldehyde solution in alcohol similarly applied.

In agreement with literature references, numerous lung tumors in mice were produced when intraperitoneal injection (I. P.) of ethyl carbamate was continued over a 13-week period. Similar effects were observed with monomethylol and dimethylol ethyl carbamate injections, although the number of tumors was less. However, no tumors were observed when methyl carbamate, monomethylol methyl carbamate, dimethylol methyl carbamate, 2-hydroxyethyl carbamate, dimethylol hydroxyethyl carbamate, and dimethylol isopropyl carbamate were similarly tested. The results were compared with those obtained by I. P. injection of some common finishing agents. No tumors were formed by ethylene urea or dihydroxyethylene urea or their dimethylol derivatives.

In a 2-year feeding study, male and female rats were fed the diets ad libitum: control diet, and the control diet with added formaldehyde, methyl carbamate, ethyl carbamate, ethyl carbamate plus free formaldehyde, dimethylol methyl carbamate, and dimethylol ethyl carbamate. Data are presented on the male and female groups fed each diet to show weight gains, mean cumulative food consumption, mortality and mammary tumor incidence, hematological measurements, mean white cell differential counts, and relative organ weights. The results of histopathalogical examinations are presented and discussed. In agreement with published data, the diets containing ethyl carbamate showed greater general toxicity and enhancement of mammary tumor formation. The toxicity of dimethylol ethyl carbamate appeared to be somewhat less than that of ethyl carbamate; however, enhancement of mammary tumor formation did occur. The pathology observed for rats on diets containing methyl carbamate and its dimethylol derivative was undistinguished from that of the controls so that methyl carbamate and its derivatives may be considered to be relatively harmless. Tumor formation from diets containing methylolated ethyl carbamates is probably due to regeneration of ethyl carbamate in the animal body.

INTRODUCTION

Dimethylol monoalkyl carbamates as textile finishing agents were developed at the Southern Regional Research Laboratory in 1961, and have since been used in treatments for wash-wear and durable press garments (1, 2, 9, 10, 11, 12, 13, 19, 20).[1/] The carbamate agents were accepted by the textile industry principally because of the

1/ Underscored figures in parenthesis refer to Literature Cited at end of publication.

excellent durability of the finish to abusive laundering conditions, particularly to excessive bleaching with hypochlorite in the home and to the highly acid "souring" used by many commercial laundries. The latter treatment leads to hydrolytic removal of most wash-wear agents with concomitant shrinkage of the garment. Use in textile finishing in this country has grown rapidly to about 4 million pounds per year in 1965, mainly for durable press white shirts. Carbamate finishing agents were used especially because of little tendency to yellow during the cure. Six carbamates are being offered commercially for the manufacture of finishing agents. The agents are the dimethylol derivatives of methyl, ethyl, isopropyl, hydroxyethyl, hydroxypropyl, and methoxyethyl carbamates.

Besides the use of the dimethylol carbamates for finishing of cotton, there is a wide use of carbamate derivatives in other fields. Literally tons of long-chain carbamates and carbamate derivatives are used in commercial products.

After introduction of the carbamate finishing agents, the authors noted that ethyl carbamate is said to be a carcinogen. There was, of course, no record of pharmacological studies on the new dimethylol derivative. The small amount of information available showed that slight changes in chemical structure generally lessened the carcinogenicity of ethyl carbamate. Methyl carbamate was harmless and isopropyl carbamate was only one-twentieth as active as ethyl carbamate (14). Although, it seemed reasonable to assume that the methylolated carbamates would have reduced activity, the possibility that the metabolic processes could regenerate the original carbamate or some other toxic compound had to be considered. Three procedures were used to evaluate the toxicity of the dimethylol carbamates in dermal (rabbit skin and eye irritation) tests, lung tumor incidence in mice by weekly intraperitoneal (I. P.) injection, and carcinogenicity in rats by long-term (2-year) feeding tests. The present paper describes the results obtained from these studies. A brief preliminary report has been published (5).

BACKGROUND

A large number of compounds are suspect as possible carcinogens. These range from rare and unusual compounds such as diazomethane, through some of the components of cigarette smoke, and on to common organic materials such as carbon tetrachloride and dioxane. In some cases rather large quantities and continuous applications are required to initiate tumors in experimental animals, while in other cases almost incredibly small amounts are needed. As an example of the latter, as little as 1 microgram of aflatoxin B_1 may induce hepatoma in a rainbow trout. (Aflatoxin B_1 is a carcinogen produced by a common mold, (Aspergillus flavus) and has been identified as a contaminant of numerous substrates.)

An informative article by Weisburger and Weisburger (24) describes some of the cancer-inducing hazards of chemicals which the chemist may encounter in his work. Since practically all carcinogens found to date combine with proteins, textile chemists should be especially careful in handling chemicals that are reactive with cellulose, because most agents that react with cellulose react even more readily with proteins. Butadiene diepoxide is an example of a crosslinking agent which is an extremely dangerous carcinogen.

The problem of testing for carcinogenic activity is complicated by numerous chemical and biological factors. A carcinogenic combination is produced sometimes when an ordinarily innocuous chemical is activated by an initiator which, by itself, also produces no tumors. Furthermore, there are a multiplicity of conditions that may affect carcinogenicity. Metabolic processes may change a harmless chemical into a dangerous one. Results may vary with species and occasionally with the physical condition of the animal. Where a large dose may cause tumors, a small dose may be harmless or even cause regression of a tumor in some cases.

The serious problem arises of exposure to possible toxic effects from various finishing agents, including crosslinking agents, dyestuffs, reactive softeners, and light reactive compounds. Testing of the agents is complicated, difficult, and expensive. In most cancer research, bioassay is performed

using either the mouse or the rat as the test animal. Potent carcinogenic compounds are easily identified but the less powerful agents may require a large number of test animals and even then the results may be in doubt. The Weisburgers (24) stated that a compound giving 30-percent incidence of tumors in rats or mice would be a relatively weak carcinogen and one giving a 5-percent incidence would be dubious.

Until 1964, the possible toxic effects of crosslinking finishing agents were of concern mainly to the textile chemist and the finisher who had to handle them. These people were familiar with the precautions necessary in applying dangerous materials to fabric, such as mercerizing strength caustic, formaldehyde, and pyridinium compounds. The garment manufacturer and the eventual user of the garment were exposed only to the small amounts of residual agents left on the fabric in those cases where no afterwash was employed. In 1964, however, durable press garments (or "permanent press," as they are termed in the advertisements) suddenly swept the textile markets. This has been described as the most revolutionary development in textiles since the introduction of nylon. In the deferred cure process for producing durable press, the crosslinking agent with catalyst and softener is applied to fabric and the latter dried but not cured. The fabric is manufactured into garments and then cured. This development greatly increased the number of people who might be in contact with textile finishing agents. An immediate problem was the profuse, irritating vapors of formaldehyde which were released from the uncured fabric during cutting and sewing.

Separate from irritation of mucous membranes by formaldehyde vapors is allergy to free formaldehyde or to other portions of the finishing agents. Such allergies have been reported (17) in a few people, and these people obviously cannot work with delayed cure cloth. However, after 2 years of manufacturing millions of garments in all parts of the country, it is obvious that the various irritants are being tolerated without, apparently, any serious effects.

Possible tumor formation is a more serious problem and consideration must be given to possible effects of the crosslinking agents used.

PREVIOUS PHARMACOLOGICAL WORK WITH CARBAMATES

The following describes a few pertinent references to indicate the scope of investigations on the pharmacological effects of carbamates. Despite the work that has been carried out, the mechanism of carbamate reactions in vivo is still a matter of speculation. Thus, it was not possible to predict with confidence whether methylolation of the monoalkyl carbamates would change their toxicological characteristics. From the brief literature review, it was evident that toxicological investigations of the new dimethylolated carbamate finishing agents would be of interest to the textile chemist and should be performed.

On the optimistic side it has been found (14) that methyl and beta-hydroxyethyl carbamates (4) are not carcinogenic when injected into rats and mice. On the other hand, ethyl carbamate produced tumors in mice, rats, and hamsters, although not in guinea pigs or chickens (7). Isopropyl carbamate was found to be approximately one-twentieth and N-propyl carbamate one-eightieth as active a carcinogen as the ethyl compound. There appears to be little agreement on the mechanism by which ethyl carbamate may cause production of a carcinoma. As late as January, 1966, Schoental (21) had proposed a mechanism by which N-hydroxyethyl carbamate, N-alkyl-N-nitroso ethyl carbamate, and similar compounds may act. However, with this mechanism it is not clear why there should be a great difference between methyl and ethyl carbamate.

Ethyl carbamate, under its common name urethane, was dispensed as a tranquilizer for a number of years until it was displaced by the several long-chain carbamates which are now used. Although it is one of the few compounds now classed as a carcinogen which has been thoroughly "tested" on human beings, apparently no records are available as to its effect with regard to formation of tumors.

Ethyl carbamate has a place in the treatment of cancer. Paterson and coworkers (18)

in 1946 found the palliative effect of ethyl carbamate to be very great in many cases. Loge and Rundles, in 1949 (15), treated multiple myeloma and observed a "striking benefit relating to all aspects of the disease." An interesting point is that doses of from 120 to 290 grams were given to each person over a 7- to 13-month period.

Some investigators have obtained beneficial synergistic effects by using ethyl carbamate with a second treatment, such as with the nitrogen mustards (Sorokina, 22, and Armaghan, 3). A considerable amount of effort has gone into attempts to determine the mechanism by which ethyl carbamate may act. Berenblum and coworkers in 1959 (4) surveyed a number of derivatives and analogs of ethyl carbamate for both lung carcinogenesis and skin tumor initiation in mice. They were, however, unable to locate a possible metabolite that might be implicated in the action on the body. Ethyl carbamate remained the most powerful of the many compounds tested. In 1962 Tannenbaum and coworkers (23) elaborated previous work on the effect of ethyl carbamate by using it on rats which were allowed to live out their life-span (about 2 years). They showed that ethyl carbamate exhibited multipotential carcinogenicity in the rat. That is, several different types of tumors were found over those found in the untreated controls.

Boyland and Nery (6) in 1965 stated that when rats, rabbits, and men are given ethyl carbamate, they excrete N-hydroxyethyl carbamate and N-acetyl-N-hydroxyethyl carbamate. N-hydroxyethyl carbamate has been shown to have carcinogenic activity of the same order of magnitude as ethyl carbamate when tested on mice. It was interesting to speculate whether introduction of one or two methylol groups, as in the monomethylol and dimethylol ethyl carbamate finishing agent, would prevent the formation in the animal of the N-hydroxyethyl carbamate.

From the results to be presented in this paper, it is evident that methylolation of ethyl carbamate has so modified its structure that its carcinogenic action has been greatly diminished. It would be interesting to determine if the excretion of N-hydroxyethyl carbamate by animals occurs only with ethyl carbamate and not with monomethylol ethyl or dimethylol ethyl carbamate. The role of the N-hydroxy derivative of ethyl carbamate as a carcinogen has been challenged by Mirvish (16). He concluded that N-hydroxyethyl carbamate is more likely to act as a carcinogen by way of conversion into ethyl carbamate itself, rather than the reverse as suggested by other workers. On this basis one might predict that any derivative of ethyl carbamate would possess carcinogenic properties if the ethyl carbamate were regenerated from the derivative during its metabolism in the animal body.

EXPERIMENTAL AND RESULTS

Rabbit Skin and Eye Irritation Tests

The official Food and Drug Administration (FDA) test for possible primary skin irritation makes use of a patch-test technique on the intact skin of the albino rabbit, clipped free of hair. The technique consists of applying a one-inch cotton patch to the skin. The patch consists of a treated cotton cloth per se, or of untreated cotton patches to which a solution of the test material is added. The rabbits are immobilized in an animal holder with the patches secured in place by adhesive tape. The entire trunk of the animal is then wrapped with rubberized cloth for the 24-hour period of exposure. After 24 hours, the patches are removed, and the results are evaluated on the basis of a scoring system which takes into account the degree of irritation (erythema) and the degree of swelling (edema) produced. Readings are also made after 72 hours and these results are included in the evaluation of effects.

Based on the scoring system shown at the bottom of table 1, it was found that dimethylol ethyl carbamate, dimethylol methyl carbamate, and dimethylol hydroxyethyl carbamate were moderate skin irritants when 50-percent alcoholic solutions of these compounds were applied to the patches several times during the test period. Completely negative results were obtained when tests were conducted with treated cotton cloth, either before or after curing. The cloth was treated by conventional finishing procedures (19) with a 10-percent solution of the dimethylol carbamate containing 0.4 percent zinc nitrate catalyst. The cloth was not washed before testing.

Because all cloth samples tested for primary skin irritation were negative, the data have not been tabulated. The results in table 1 indicate the approximate degree of irritation produced when half milliliter portions of 50 percent alcoholic solutions of the various carbamates were singly applied to untreated cloth patches which were held in direct contact with the skin for 24 hours. Erythema or redness but no edema (swelling) was observed under these conditions.

Apparently the quantity of chemical actually making contact with the skin from a treated cloth patch is below the threshold level required to produce visible irritation, in contrast to the positive action produced when the chemicals are applied directly to the patch in a higher concentration. Another factor that obviously influences the extent of the irritation produced is the solvent and humidity (perspiration) relationships which prevail during the period of skin contact of the material being tested. For example, when three daily applications of dimethylol ethyl carbamate (0.25 cc of a 50 percent alcoholic solution) were applied directly to the skin of a rat, there was no evidence of skin irritation, in contrast to the irritant effects on rabbit skin.

The effects of ethyl carbamate, dimethylol ethyl carbamate, and dimethylol methyl carbamate on the eye mucosa of albino rabbits were also investigated. Following the direct instillation into the eye of these compounds in aqueous solution, inflammation of the eye mucosa was evident for ethyl carbamate and dimethylol ethyl carbamate, whereas there was no apparent irritation resulting from the instillation of dimethylol methyl carbamate.

Mouse Lung-tumor Tests

The bioassay for mouse lung-tumor incidence was conducted using the procedure described by Larson (14). In brief, healthy mice, 10 to 12 weeks of age, were given the test substances by intraperitoneal injection once weekly for 13 successive weeks. All mice were subsisted on a commercial mouse diet and water ad lib. Two to three weeks after the thirteenth injection each mouse was sacrificed and examined for gross pathological changes. The lungs were excised and immediately examined under a dissecting microscope for the occurrence and number of peripheral pulmonary tumors. The results of trial 1 are summarized in table 2. No lung tumors were detected in the group of six strain A tumor susceptible mice which served as controls and received injections of water only. According to Larson (14), 17 percent of strain A mice, or one out of six, should have a single lung tumor (0.18 tumors per mouse). As expected, both the strain A mice and the non-tumor prone Swiss-Webster mice, which were injected with a 5-percent solution of ethyl carbamate (0.01 cc. per g. body weight), developed lung tumors. The mean number of lung tumors per mouse was 44 and 38, respectively. No lung tumors were detected in the groups injected with 2-hydroxyethyl carbamate. Unfortunately, the mice receiving I. P. injections of 5 percent aqueous solutions of dimethylol hydroxyethyl, dimethylol methyl, and dimethylol ethyl carbamate failed to survive after several weekly injections; hence, no data could be obtained. In other words, the dimethylol derivatives were decidedly more toxic than the corresponding carbamate per se when comparable doses were injected.

In trial 2, the mouse lung tumor bioassay was repeated using a lower dosage (1-percent solution) of the three compounds which caused fatalities in trial 1. Because the incidence of lung tumors produced in the two strains of mice given ethyl carbamate in trial 1 was comparable (44 vs. 38), only Swiss-Webster mice were used in trial 2. The results are shown in table 3. The findings were negative except for a single lung tumor, which was observed in one of six mice injected with dimethylol methyl carbamate. The occurrence of an occasional spontaneous tumor unrelated to the treatment is reasonable to expect.

In the third mouse lung tumor assay (trial 3), eight male Swiss-Webster mice per group were given six carbamate injections (table 4). The mean number of lung tumors found per mouse are clear-cut in that, with the exception of group no. 6, no tumors were detected in contrast to the positive control group no. 3, which received ethyl carbamate. The mean number of tumors per mouse in this trial is appreciably reduced in comparison with trial 1 (6.5 vs. 38), but it should be noted that the dosage of ethyl carbamate administered was reduced from 5 percent to 2-percent solution. Unfortunately, in group

no. 6 (dimethylol ethyl carbamate injected) only 3 of 8 mice survived the series of 13 injections and 1 of the 3 survivors had 11 lung tumors, whereas the other 2 survivors had none. In trial 3, mice injected with monomethylol methyl carbamate did not develop lung tumors. This is not surprising because Larson (14) had reported earlier that methyl carbamate in contrast to ethyl carbamate did not produce lung tumors.

In trial 4, (table 5), attempts were made to clarify the results of the previous trial (table 4) in which only three mice out of eight survived the injections of dimethylol ethyl carbamate and one of the three survivors had lung tumors. The results indicate that dimethylol ethyl carbamate does produce lung tumors in mice when a 2-percent solution was used, in that four of the eight mice had a total of five tumors. The development of tumors appeared to be dosage dependent in that there was only one tumor in one mouse out of eight injected with a 1-percent solution of dimethylol ethyl carbamate. The importance of dosage is also shown by comparing the lung tumor incidence for ethyl carbamate in tables 2, 4, and 5, in which the concentrations of the solutions of ethyl carbamate injected were 5, 2, and 2 percent, respectively and the lung tumor incidence was 38, 6.5, and 3.5 tumors per mouse, respectively. It is also interesting to note (table 5) that the incidence of lung tumors for mice injected with monomethylol ethyl carbamate was midway between ethyl carbamate and dimethylol ethyl carbamate (5 of 8 mice had 11 lung tumors). Also, Mirvish (16) concluded that N-hydroxyethyl carbamate acts as a carcinogen by virtue of its conversion into ethyl carbamate. It seems probable that some ethyl carbamate has been regenerated from dimethylol ethyl carbamate and monomethylol ethyl carbamate, to account for the weak mouse lung tumor action tabulated in table 5. This same argument would also account for the negative results observed for dimethylol hydroxyethyl carbamate and dimethylol methyl carbamate, because the two parent substances that are capable of being regenerated here would be hydroxyethyl carbamate and methyl carbamate, respectively, neither of which have been implicated as lung tumor producers.

The results of a mouse-lung tumor bioassay of a series of ethyleneurea derivatives, which also are used as wash-wear finishing agents, are tabulated in table 6. The results were negative in that none of the ethyleneurea derivatives produced lung tumors in mice, whereas, under the same conditions ethyl carbamate did produce tumors in mice. The results would not be unexpected, however, since ethyleneurea per se is also negative. Dimethylol ethyleneurea was unique in that a high mortality rate resulted when mice were injected with a 5-percent solution. There were no deaths in the group of mice injected with a 1-percent solution of this material.

Larson (14) also tested a series of alkyl carbamates other than ethyl (table 7). His results clearly show that the n-propyl and isopropyl derivatives were slightly active as producers of lung tumors; whereas the methyl, n-butyl, and isoamyl derivatives were inactive.

Berenblum and coworkers (4) obtained negative results when N,N-dimethylethyl carbamate was tested for mouse-lung tumor carcinogenesis; whereas the mono-substituted derivative (N-methylethyl carbamate) gave a positive test (table 8).

Two-Year Rat Feeding Study of Carbamate Derivatives

Materials and Methods

Young rats (average weight, 95 to 100 g.) from our colony were separated into 7 groups of 10 for each sex and housed 5 per cage. Animals of the same sex were distributed so that each cage contained rats with nearly the same average weight. Test diets were prepared by thoroughly mixing the compound of interest into our standard rat ration, Addis, as follows: formaldehyde in 37-percent aqueous solution was added to a level of 2.2 g./kg.; dimethylol methyl carbamate and dimethylol ethyl carbamate in 50-percent aqueous solution and methyl and ethyl carbamates in 50-percent alcohol solution were added to a level of 1 g./kg. These carbamate derivatives were prepared by one of the authors (J. David Reid): dimethylol methyl and dimethylol ethyl carbamates in 50-percent aqueous solution, and methyl and ethyl carbamates in crystalline form. The dimethylol derivatives are equilibrium mixtures of 2.5 moles formaldehyde and 1 mole ethyl or methyl carbamate, and their solutions contain excess formaldehyde (19). Therefore, Addis plus formaldehyde was used as one control. Also a diet was prepared containing ethyl carbamate mixed with

formaldehyde for comparison with a diet containing only ethyl carbamate. The seven diets fed and their abbreviations are:

Addis control	(A)
Addis + formaldehyde	(F)
Addis + methyl carbamate	(MC)
Addis + ethyl carbamate	(EC)
Addis + ethyl carbamate + formaldehyde	(EC+F)
Addis + dimethylol methyl carbamate	(DMMC)
Addis + dimethylol ethyl carbamate	(DMEC)

The basal (Addis) diet consisted of degerminated yellow cornmeal 73 percent, crude casein 10 percent, linseed oil cake meal 10 percent, ground alfalfa 2 percent, bone ash 1.5 percent, sodium chloride 0.5 percent, and cod-liver oil USP 3 percent.

Each group of rats was fed exclusively one of the test diets, ad libitum, for the term of the experiment. Weights of individual rats and food consumption per cage were measured weekly. Also noted was the general health and incidence of subcutaneous tumors.

Based on actual food consumption, the average daily ingestion of carbamate derivatives was 10.5 mg./rat for females and 12.5 mg./rat for males. This was equivalent to a daily dose initially of 110 mg./kg. for females and 119 mg./kg. for males, and by 10 weeks, of 56 mg./kg. and 51 mg./kg., respectively.

All rats, except those that were moribund, were allowed to live until 105 weeks. At autopsy, organs were examined grossly and weighed, and tissue samples were taken for microscopical examination.

At 74 weeks, white blood cell (w.b.c.) counts, white cell differential and hematocrit measurements were made, first on moribund animals followed by general surveys of entire groups. Starting at 84 weeks, hemoglobin levels were also determined. Again, just before termination of the experiment, blood from all survivors was analyzed and red blood cell (r.b.c.) counts were made.

Unfortunately, around the 85th week of feeding, a respiratory infection accompanied by high mortality swept through our entire rat colony, including the animals being used for this study.

Results

Early in the experiment, the growth curves of female rats showed consistent differences related to dietary groups. Mean body weights at 10, 40, and 80 weeks as analyzed by Duncan's multiple range F test (8) are shown as follows:

[Any two means not underscored by the same solid line are significantly different at the 5-percent level and at the 1-percent level when the dotted lines are included.]

Female:

10 wk. Diet	EC+F	EC	A	DMEC	F	MC	DMMC
Mean g.	173	174	179	181	185	186	190

40 wk. Diet	EC	EC+F	F	DMEC	MC	A	DMMC
Mean g.	220	230	240	241	244	253	263

80 wk. Diet	EC	EC+F	MC	F	DMEC	A	DMMC
Mean g.	245	267	296	303	313	320	332

Male:

10 wk. Diet	EC+F	A	DMMC	MC	EC	DMEC	F
Mean g.	241	243	244	245	245	249	256

40 wk. Diet	EC+F	MC	EC	A	F	DMEC	DMMC
Mean g.	332	344	348	355	355	357	367

80 wk. Diet	F	EC+F	A	MC	DMEC	EC	DMMC
Mean g.	361	370	398	410	415	416	420

All groups on diets containing ethyl carbamate had the lowest weight throughout the entire time of the experiment. By 10 weeks their means were significantly less than those of the 3 heaviest groups, F, MC, and DMMC ($P < 0.01$); at 40 weeks less than A and DMMC ($P < 0.05$); and at 80 weeks less than DMEC, A and DMMC ($P < 0.05$). The group of females with the greatest growth rate was DMMC which at 10 weeks was significantly larger than Addis ($P < 0.01$). This group remained the heaviest up to 105 weeks.

The growth of the male rats showed a much less consistent relationship to dietary groups. However, similar to the females, the group EC+F had the lowest weight until

40 weeks and remained relatively low for the rest of the experiment. Also, at 34 weeks, DMMC again emerged as the heaviest group and remained so. The overall range in body weights among the various groups was somewhat less than that of the females and no significant differences ever developed.

Mean cumulative food consumption at 10 and 40 weeks as analyzed by Duncan's multiple range F., is presented as follows:

[Any two means not underscored by the same solid line are significantly different at the 5-percent level and at the 1-percent level when the dotted lines are included.]

Female:

10 wk.

Diet	F	EC+F	EC	DMMC	DMEC	A	MC
Mean g./rat/day	9.96	10.00	10.11	10.31	10.52	11.27	11.48

40 wk.

Diet	F	EC	EC+F	DMMC	DMEC	MC	A
Mean g./rat/day	9.65	9.76	9.78	9.94	10.61	10.62	11.42

Male:

10 wk.

Diet	EC+F	EC	F	DMMC	A	DMEC	MC
Mean g./rat/day	11.67	11.77	11.89	12.80	12.90	13.20	13.27

40 wk.

Diet	EC+F	EC	F	DMEC	DMMC	MC,A
Mean g./rat/day	12.46	12.61	12.67	13.02	13.05	13.34

The three diets, EC, EC+F, and F, were consistently eaten in the least amount by both sexes. The difference in consumption between them and the groups A and MC was significant at the 95-percent level or better at 10 and 40 weeks. The dimethylol derivatives were consumed at intermediate levels. Some degree of correspondence between growth and food consumption can be seen for females on the two diets containing ethyl carbamate. Both values for these groups are among the lowest. The same can be said for the group of males, EC+F. However, for the other diets, no particularly consistent relationship can be found.

Table 9 shows the number of survivors at 70 and 90 weeks, the period which spans the occurrence of the respiratory infection, and at 105 weeks when the experiment was terminated. At 70 weeks, prior to the infectious disease crisis, the average mortality was 29 percent for females and 14 percent for males, with groups EC and EC+F of both sexes being among those with the greatest number of deaths. As a result of the epidemic, at 90 weeks it may be noted that these same two groups of both sexes suffered the greatest losses. At 105 weeks the mortality of male and female rats on diets containing the other carbamate derivatives were not noticeably different from the Addis or formaldehyde controls, with the exception of that for females on DMEC. The final number of male survivors was over 2.5 times that of the females.

Enhanced mammary tumor formation, both in terms of greater number and shorter time for first appearance, occurred with females on diets EC, EC+F, and DMEC (table 9). An intermediate level of tumor incidence was observed for females on diets MC and DMMC. With the males, however, tumor incidence was considerably less and without apparent relation to diet.

Averages of the last blood measurements taken before death are shown in table 10. Although some variation is seen, there is no consistent dietary relationship relative to the other effects already noted, except possibly for lowered hematocrit and hemoglobin values for females on EC. The male group with the lowest hemoglobin value is also EC. Individual white blood cell values varied greatly in apparent response to the degree of infection at any particular time. Hematocrit and hemoglobin values measured at 95 weeks and again before autopsy showed a general decrease in nearly all dietary groups, especially for the female rats. Red blood cell counts, taken of survivors at 105 weeks, were generally within normal range, except for one moribund female rat on formaldehyde which was extremely anemic. Average values of white cell differential counts taken during the latter part of the experiment are shown in table 11. All values are in the normal range for rats and no particular relation to dietary group can be seen.

Table 12 shows the organ weights of rats taken at the conclusion of the experiment calculated relative to 100 g. body weight.

Although comparison among the various groups is made difficult by the low number

of survivors, average relative weight of liver, heart, kidneys, and testis of males on the two diets containing EC are seen to be among the three largest groups. This result did not appear to be due to either consistently larger than average absolute organ weight or smaller than average body weights for these groups. DMEC was also among the three largest groups for liver and heart. There were no female survivors on the EC-containing diets, but the single survivor on DMEC had a larger relative liver weight than the average of any other dietary group.

An overall comparison to terminal body weights revealed that most organ weights, on an absolute basis, merely reflected body weight. Liver, heart, kidneys, and testis in the male and liver and kidneys in the female were highly correlated to body weight ($P<0.01$). Spleen weights varied greatly and were quite unrelated to body weight for both sexes.

Histopathology

Macroscopic and microscopic lesions occurred in several body organs. The lesions were inflammatory, degenerative, and proliferative (nonneoplastic) or neoplastic in character. Nonneoplastic lesions were, except as outlined, nonspecific in origin and represent normal histopathologic abnormalities occurring in old rats.

Nonneoplastic lesions occurring in the liver included centrilobular lipidosis and zonal and focal necrosis. These lesions were generally more severe in females and were present in all groups of rats. Proliferation of bile ducts with hyalinization also occurred in the livers from each group.

Sterine lesions of nonneoplastic nature include a mild endometrial fibrosis, cystic endometrial hyperplasia, and a suppurative endometritis. The metritis was often severe and contributed to, or caused death. Endometritis occurred frequently in the EC females, (50 percent), and in the EC+F females (40 percent), but less often in the DMEC females (20 percent). Endometritis was noted in only one of the remaining female rats of all groups. The lesion is apparently directly or indirectly related to the test compound utilized.

A variety of lesions, including irregular enlargement of muscle fibers and nuclei, bizarre-shaped nuclei, mild sporadic fibrosis, and focal accumulation of mononuclear cells, was present in the myocardium (heart). These lesions were present in rats of all groups, with overlap in degree between various groups. One or more rats in each group had changes more severe than those seen in the control rats with the EC males demonstrating prominent changes most consistently.

The significance of the myocardial alterations is difficult to assess due to the overlap between the various groups. The lesions are probably not directly related to the treatment as similar lesions have been recognized in hearts of individuals suffering from diseases such as rheumatic fever.

Neoplastic lesions of significance occurred in the mammary gland. Most tumors, 24 studied microscopically, were benign fibroadenomas. Two tumors were malignant and were adenocarcinomas. The incidence of mammary tumors was 30, 35, and 35 percent in the EC groups and the DMEC group, respectively. The incidence was 25 percent in the F groups, 30 percent in the MC group, 30 percent in the DMMC group, and 25 percent in the control group. The significance of these tumors rests more with the time of appearance perhaps than the incidence of occurrence.

A benign tumor of salivary gland tissue occurred in the EC group and the EC+F group. A malignant tumor of salivary gland origin also occurred in the latter group. All three tumors were in female rats.

Two malignant tumors (adenocarcinomas) occurred in the EC male rat group. One was located in the stomach and the other in the intestine. An adenocarcinoma was present in the stomach of a male rat in the EC+F group.

The salivary tumors and the gastrointestinal tumors are difficult to evaluate because of the small number of animals utilized and the absence of a greater number of tumors. These tumors, however, were not observed in other groups of rats.

Other tumors including five pituitary adenomas, twenty adrenal adenomas, (cortical and medullary), a transitional cell tumor of the urinary bladder, two bronchogenic carcinoma, and a metastic fibroma were noted. These tumors did not demonstrate any significant group specificity.

Discussion of Rat Feeding Studies

For the various physiological indices of health followed in this 2-year feeding study, the female rats showed a wider range of response and greater sensitivity to diet than did the males. Growth was significantly depressed for females on the two diets containing ethyl carbamate and to a lesser extent for males on only one of these diets. This lowered growth rate may be partly due to decreased food consumption of these diets, although the relationship between food consumption and growth was inexact. For example, even though the diet containing formaldehyde was among the least consumed, it was not particularly associated with the lowest growth rates. Rats of both sexes appeared to have the largest growth rate on diet DMMC. However, the significance of this is not known because this diet was never among the highest consumed. The group fed with the dimethylol derivative of ethyl carbamate was not particularly distinguished in either growth or food consumption.

With respect to mortality, the female rats of all groups were less hardy than the males, which survived the females at better than 2.5 to 1. However, both sexes suffered a distinctly greater death rate on the two diets containing ethyl carbamate. Some indication of toxicity for DMEC was associated with an intermediate death rate for female rats.

Enhancement of mammary tumor production in the females in terms of both increased incidence and shortened time for the first appearance resulted from feeding ethyl carbamate. This agrees with the findings of Tannenbaum and coworkers (23), although a net increase in tumor incidence could not be demonstrated because of the high mortality associated with the more than two times greater dosage of ethyl carbamate. They also found a reduction in the latent period for tumor formation in male rats, however, the incidence was considerably less than for females. The occurrence of tumors in the male rats was about one-half of that for the females but without any association to diet.

DMEC was linked with enhanced mammary tumor formation in females to the same extent as was the ethyl carbamate-containing diets.

No clear relation to diet was shown by the various hematological measurements, although individual animals that were moribund often showed evidences of anemia. Also, with advancing age, the general trend was toward anemia. However, average values of all groups remained within accepted normal limits.

Absolute organ weights also revealed no information related to diet but were found to be closely associated to body weight, with the exception of the spleen. Calculation of organ weight relative to body weight showed some indication of increase, again with the exception of the spleen, associated with the two diets containing ethyl carbamate and possibly the dimethylol derivative of ethyl carbamate.

Microscopical examination of tissues revealed similar relationships among the various diets as already noted. Again the females displayed greater adverse effects to the conditions of the experiment by the occurrence of more severe nonneoplastic lesions of the liver. The toxicity of diets containing ethyl carbamate was also evident upon tissue examination. In the females, these diets were associated with the uterine lesion, endometritis, and the incidence of three tumors of the salivary gland. In the males, the prominence of a variety of myocardial lesions and the presence of two malignant gastro-intestinal tumors implicated these diets.

The incidence of mammary tumors and endometritis placed dimethylol ethyl carbamate in a position of intermediate toxicity. Dimethylol methyl carbamate was not associated with any increased pathology over that of the controls.

SUMMARY

Eight solutions of carbamates, or the methylolated derivatives of the type used as textile finishing agents, were evaluated as rabbit skin irritants. No evidence of skin irritation was found when treated cotton cloth was patch-tested on rabbit skin. However, when 50 percent alcoholic solutions were applied for 24 hours, methyl carbamates and

dimethylol methyl, dimethylol ethyl, and hydroxyethyl carbamates were all moderate skin irritants about equal in intensity to that of a 5-percent formaldehyde solution in alcohol.

Some carbamates and carbamate derivatives were evaluated toxicologically as producers of lung tumors in mice after intraperitoneal injection over a 13-week period. Lung tumors were produced in mice injected with ethyl carbamate, monomethylol ethyl carbamate, and dimethylol ethyl carbamate, whereas no tumors were observed when methyl carbamate, monomethylol methyl carbamate, dimethylol methyl carbamate, 2-hydroxyethyl carbamate, dimethylol hydroxyethyl carbamate, and dimethylol isopropyl carbamate were similarly tested. Some ethyleneurea derivatives were tested also. No tumors were observed with ethyleneurea, dimethylol ethyleneurea, dihydroxy ethyleneurea or dimethylol dihydroxy ethyleneurea.

In a 2-year feeding experiment with rats, the dimethylol derivatives of methyl and ethyl carbamate and their parent compounds were fed at the rate of 0.1 percent of the diet. Growth, food consumption, mortality, hematology, and macroscopic and microscopic tissue pathology were noted. The positive control diets, those containing ethyl carbamate, showed greater general toxicity and enhancement of mammary tumor formation. The toxicity of dimethylol ethyl carbamate appeared to be somewhat less than that of ethyl carbamate; however, enhancement of mammary tumor formation did occur. The pathology observed for rats on diets containing methyl carbamate and its dimethylol derivative was undistinguished from that of controls.

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Table 1. --Rabbit Skin Irritation Study of Carbamates

Substance tested[1/]	Erythema score	Edema score	Total score[2/]
Formaldehyde (5 percent in EtOH)	2	1	3
Ethyl carbamate	0	0	0
Methyl carbamate	2	1	3
2-hydroxyethyl carbamate	0	0	0
Monomethylol ethyl carbamate	0	0	0
Dimethylol ethyl carbamate	3	0	3
Dimethylol methyl carbamate	2	0	2
Dimethylol hydroxyethyl carbamate	2	0	2
Dimethylol isopropyl carbamate	2	0	2

1/ 50-percent solution in ethyl alcohol unless indicated otherwise.

2/ Interpretation of total score according to J.H. Draize, formerly Chief, Skin Toxicity Branch, FDA.

Score	Degree of irritation
0	No irritation
<2	Mild irritation
2-5	Moderate irritation
>6	Severe irritation

Table 2. --Trial 1: Mouse Lung-Tumor Bioassay of Strain A and Swiss-Webster Male Mice

Group No.	Carbamate injected	Mouse strain [1]	Survivors [2] at 13 weeks	Mean lung tumors per mouse
			number	number
1	Control (water only)	A	6	0
2	2-hydroxyethyl	A	6	0
3a	Dimethylol hydroxyethyl	A	2	0
3b	Dimethylol hydroxyethyl	SW	0	-
4	Dimethylol methyl	A	0	-
5a	Dimethylol ethyl	A	0	-
5b	Dimethylol ethyl	SW	0	-
6a	Ethyl carbamate	A	6	44
6b	Ethyl carbamate	SW	6	38

1/ Strain A mice are tumor susceptible; strain SW are conventional Swiss-Webster male mice.

2/ Six mice used per group, each survivor received 13 weekly I.P. injections of 0.01 ml./g. body weight of a 5-percent solution in water.

Table 3. --Trial 2: Mouse Lung-Tumor Bioassay of Swiss-Webster Female Mice

Group No.	Carbamate injected	Survivors [1] at 15 weeks	Mean lung tumors per mouse
		number	number
1	Dimethylol hydroxyethyl	6	0
2	Dimethylol methyl	6	0.16(?)
3	Dimethylol ethyl	4	0

1/ Six mice used per group; injected once weekly for 13 weeks with 0.01 ml./g. body weight of a 1-percent solution in water.

Table 4. --Trial 3: Mouse Lung-Tumor Bioassay of Swiss-Webster Male Mice

Group No.	Carbamate injected	Survivors [1] at 16 weeks	Mean lung tumors per mouse
		number	number
1	Control (water only)	8	0
2	Dimethylol methyl	7	0
3	Ethyl carbamate	8	6.5
4	Dimethylol isopropyl	8	0
5	Monomethylol methyl	8	0
6	Dimethylol ethyl	3	3.7 (all in one mouse)

1/ Eight mice used per group; injected once weekly for 13 weeks with 0.01 ml./g. body weight of a 2-percent solution in water.

Table 5. --Trial 4: Mouse Lung-Tumor Bioassay

Carbamate injected	Dosage	Survivors [1] at 16 weeks	Total lung tumors	Mean lung tumors per mouse	Mice with no lung tumors
	Percent	number	number	number	number
Control (water only)	-	8	0	0.0	8
Ethyl carbamate	2	8	27	3.5	0
Dimethylol ethyl	1	7	1	.1	6
Dimethylol ethyl	2	8	5	.6	4
Monomethylol ethyl	2	8	11	1.4	3
Dimethylol hydroxyethyl	2	8	0	0	8

1/ Eight mice per group; 13 weekly I.P. injections of 0.01 ml./g. body weight of a 1- or 2-percent solution in water as indicated.

Table 6. --Mouse Lung-Tumor Bioassay of Ethylene Urea Derivatives

Compound injected	Concentration	Survivors [1]	Mean number tumors per mouse
	Percent	number	
Ethyl carbamate	5	8	16 [2]
Ethyleneurea	5	8	0
Dimethylol ethyleneurea	1	8	0
Dimethylol ethyleneurea	5	3	0
Dihydroxy ethyleneurea	5	8	0
Dimethylol dihydroxy ethyleneurea	5	8	0

1/ Eight mice used per group; each mouse received 13 weekly I.P. injections of 0.01 ml./g. body weight of indicated concentration in water

2/ Range 7 to 27.

Table 7. --Mouse Lung-Tumor Induction by Esters of Carbamic Acid [1]

Compound	Dose per mouse mg./g.	Mice [2]	Mice with tumors	Mean lung tumors per mouse
		number	Percent	number
Control	-	141	17	0.18
Methyl	0.5	46	16	.19
Ethyl	.5	102	100	76.9
n-propyl	.5	78	63	.95
Isopropyl	.5	51	90	3.7
n-butyl	.25	33	12	.12
Isoamyl	.5	23	13	.13

1/ Data from C. D. Larsen (14).

2/ Strain A mice, 10 to 12 weeks of age; intraperitoneal injection once per week for 13 weeks, autopsied 2 to 3 weeks after final injections.

Table 8. --Skin Initiating Action and Lung Carcinogenesis in Swiss Mice 2 to 3 Months of Age by a Single Intraperitoneal Injection or Oral Administration of Urethane Derivatives 1/

(Results after 40 weeks (80 applications) of subsequent croton oil treatment.)

Compound	Initiating action on skin	Carcinogenic action on the lungs
Ethyl carbamate	5+	5+
N-methylethyl carbamate	2+	2+
N, N-dimethylethyl carbamate	0	0
N-hydroxyethyl carbamate	3+	2+
Propyl carbamate	1+	1+
Allyl carbamate	1+	0
β-hydroxyethyl carbamate	$\pm$	0

1/ Data from I. Berenblum (4).

Table 9. --Survival and Mammary Tumor Incidence of Rats from Two-Year Feeding Study 1/

Group and diet	Survivors weeks on diet			Rats with mammary tumors weeks on diet		
	70	90	105	70	90	105
	Number	Number	Number	Number	Number	Number
Female:						
A	8	5	2	0	2	2
F	6	3	2	0	1	1
MC	7	4	4	0	2	4
EC	5	0	0	3	5	5
EC+F	7	0	0	1	6	6
DMMC	9	5	4	0	1	4
DMEC	8	3	0	2	5	6
Male:						
A	10	7	5	0	2	3
F	9	7	7	0	0	4
MC	7	5	4	0	1	2
EC	7	3	2	0	0	1
EC+F	8	2	2	0	1	1
DMMC	9	5	5	1	1	2
DMEC	10	8	6	0	0	1

1/ Ten mice used per group.

Table 10. --Hematological Measurements of Rats
(Average of last values before death or autopsy)

Group and diet	White blood cells x $10^{-3}/mm^3$	Hemato-crit	Hemo-globin	Group and diet	White blood cells x $10^{-3}/mm^3$	Hemato-crit	Hemo-globin
Female		Pct.	g. Pct.	Male		Pct.	g. Pct.
A	20	37	9.8	A	17	43	13.7
F	15	36	9.9	F	17	48	15.6
MC	12	46	14.2	MC	16	42	14.3
EC	17	29	6.4	EC	16	43	11.5
EC+F	12	40	11.5	EC+F	23	45	12.3
DMMC	10	43	13.9	DMMC	17	43	13.9
DMEC	11	43	13.6	DMEC	13	40	11.9

Table 11. --Mean White Cell Differential Counts of Rats

Group and diet	Neutrophiles	Lymphocytes	Monocytes	Eosinophiles	Basophiles
Female:	Percent	Percent	Percent	Percent	Percent
A	38.9	57.0	2.6	1.3	0.3
F	34.2	60.7	2.7	2.2	.3
MC	37.5	58.2	2.5	1.6	.2
EC	36.7	58.4	3.0	1.3	.5
EC+F	34.1	62.2	2.2	1.0	.5
DMMC	36.0	59.6	2.6	1.4	.3
DMEC	35.4	60.5	2.4	1.4	.3
Male:					
A	36.6	57.8	2.8	2.5	0.2
F	38.0	57.6	2.6	1.4	.3
MC	44.6	51.1	2.4	1.5	.3
EC	39.5	55.9	2.2	2.0	.4
EC+F	41.3	54.2	2.4	1.9	.2
DMMC	34.1	61.7	2.9	1.2	.1
DMEC	34.1	60.9	2.7	1.9	.4

Table 12. --Relative Organ Weight of Rats

Group and diet	Survival of animals	Body weight of --				
		Liver	Heart	Kidneys	Spleen	Testis
Female:	Number	g./100g.	g./100g.	g./100g.	g./100g.	g./100g.
A	2	3.37	0.353	0.779	0.324	
DMMC	5	3.71	.309	.677	.154	
DMEC	1	3.89	.297	.769	.185	
F	2	3.14	.410	.700	.319	
MC	4	3.41	.314	.666	.294	
EC+F	0	--	--	--	--	
EC	0	--	--	--	--	
Male:						
A	6	2.70	.351	.798	.298	0.720
DMMC	5	2.70	.315	.773	.215	.839
DMEC	6	3.22	.353	.844	.161	.855
F	7	2.78	.302	.764	.355	.713
MC	4	3.02	.338	.853	.177	.922
EC+F	2	3.21	.396	.852	.230	.871
EC	2	3.39	.382	.941	.244	.900

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